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पशु आहार सामग्री — मुक्त एवं सम्पूर्ण गौसीपोल की मात्रा का निर्धारण

Indian Standard

ANIMAL FEEDING STUFFS — DETERMINATION OF FREE AND TOTAL GOSSYPOL

ICS 65.120

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BUREAU OF INDIAN STANDARDS
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NEW DELHI 110002

October 2007

Price Group 2

Livestock Feeds, Equipment and Systems Sectional Committee, FAD 5

NATIONAL FOREWORD

This Indian Standard which is identical with ISO 6866 : 1985 'Animal feeding stuffs — Determination of free and total gossypol' issued by the International Organization for Standardization (ISO) was adopted by the Bureau of Indian Standards on the recommendation of the Livestock Feeds, Equipment and Systems Sectional Committee and approval of the Food and Agriculture Division Council.

The text of ISO Standard has been approved as suitable for publication as an Indian Standard without deviations. Certain conventions are, however, not identical to those used in Indian Standards. Attention is particularly drawn to the following:

a) Wherever the words 'International Standard' appear referring to this standard, they should be read as 'Indian Standard'.

b) Comma (,) has been used as a decimal marker in the International Standards, while in Indian Standards, the current practice is to use a point (.) as the decimal marker.

In this adopted standard, reference appears to the following International Standard for which Indian Standard also exists. The corresponding Indian Standard, which is to be substituted in its place is listed below along with its degree of equivalence for the edition indicated:

International Standard	*Corresponding Indian Standard*	*Degree of Equivalence*
ISO 6498 : 1998 Animal feeding stuffs — Preparation of test sample	IS 14831 : 2000 Animal feeding stuffs — Preparation of test sample	Identical

The technical committee responsible for the preparation of this standard has reviewed the provisions of following International Standard referred in this adopted standard and has decided that it is acceptable for use in conjunction with this standard:

International Standard	*Title*
ISO 6497 : 2002	Animal feeding stuffs — Sampling

In reporting the results of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'.

Indian Standard

ANIMAL FEEDING STUFFS — DETERMINATION OF FREE AND TOTAL GOSSYPOL

1 Scope

This International Standard specifies a method for the determination of the content of free and total gossypol and chemically related substances in animal feeding stuffs.

2 Field of application

The method is applicable to cottonseed and cottonseed meals and cakes, and to compound feeding stuffs containing these substances.

The detection limit for free gossypol is 20 mg/kg and that for total gossypol is 50 mg/kg.

3 References

ISO 6497, *Animal feeding stuffs — Sampling.*[1]

ISO 6498, *Animal feeding stuffs — Preparation of test samples.*

4 Principle

Extraction of the gossypol in the presence of 3-aminopropan-1-ol, either with a mixture of 2-propanol and hexane for the determination of free gossypol or with dimethylformamide for the determination of total gossypol. Conversion of the gossypol into gossypol-dianiline using aniline. Measurement of the absorbance at the wavelength of maximum absorbance (between 435 and 445 nm).

5 Reagents

All reagents shall be of recognized analytical grade. The water used shall be distilled water or water of at least equivalent purity.

5.1 2-Propanol/*n*-hexane, 60 + 40 mixture by volume.

5.2 Solvent A.

Place in a 1 000 ml volumetric flask approximately 500 ml of the 2-propanol/hexane mixture (5.1), 2 ml of 3-aminopropan-1-ol, 8 ml of glacial acetic acid and 50 ml of water. Make up to volume with the 2-propanol/hexane mixture.

This reagent is stable for one week only.

5.3 Solvent B.

Place in a 100 ml volumetric flask 2 ml of 3-aminopropan-1-ol and 10 ml of glacial acetic acid. Cool to room temperature and make up to volume with *N*,*N*-dimethylformamide.

This reagent is stable for one week only.

5.4 Aniline.

If the absorbance determined in the blank test exceeds 0,022, distil the aniline over zinc dust, discarding the first and last 10 % fractions of distillate. Store in a stoppered brown glass bottle in a refrigerator (0 to 4 °C).

The reagent is stable for several months.

6 Apparatus

Usual laboratory apparatus and in particular

6.1 Tumbler mixer, having a rotational frequency of approximately 35 min^{-1}, or other shaker.

6.2 Spectrometer, suitable for making measurements at wavelengths between 435 and 445 nm, with cells of optical path length 10 mm.

6.3 Volumetric flasks, of capacities 25 and 50 ml.

6.4 Conical flasks, of capacities 50, 100 and 250 ml.

7 Sampling

Take the laboratory sample in accordance with ISO 6497.

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[1] At present at the stage of draft.

8 Procedure

8.1 Preparation of the test sample

Prepare the test sample in accordance with ISO 6498.

8.2 Test portion

The mass of the test portion depends on the expected gossypol content. For the determination of free gossypol in cottonseed and cottonseed meals and cakes the mass of the test portion should not exceed 1 g; for compound feeding stuffs, it may be as much as 5 g. For the determination of total gossypol, the mass of the test portion should be between 0,5 and 5 g.

NOTE — The mass of the test portion should be large enough to give the quantity of free gossypol indicated in 8.3.1.2 or the quantity of total gossypol indicated in 8.3.2.4 in each aliquot portion of the filtrate.

8.3 Determination

8.3.1 Determination of free gossypol

8.3.1.1 Transfer the test portion (8.2) to a 250 ml conical flask, the bottom of which has been covered with glass beads. Add, by means of a pipette, 50 ml of solvent A (5.2), stopper the flask and mix, using the tumbler mixer (6.1), for 1 h. Filter through a dry filter paper, collecting the filtrate in a 100 ml conical flask. During filtration, cover the funnel with a watch-glass.

8.3.1.2 Transfer, by means of a pipette, identical aliquot portions of the filtrate, containing 50 to 100 µg of gossypol, to each of two 25 ml volumetric flasks (6.3) (C and D). If necessary, make up to 10 ml with solvent A (5.2).

8.3.1.3 Make up the contents of flask C to volume with the 2-propanol/hexane mixture (5.1) and mix.

This solution will be used as the reference solution against which the test solution will be measured.

8.3.1.4 Transfer, by means of a pipette, 10 ml of solvent A (5.2) into each of two 25 ml volumetric flasks (E and F).

8.3.1.5 Make up the contents of flask E to volume with the 2-propanol/hexane mixture (5.1) and mix.

This solution will be used as the reference solution against which the blank test solution will be measured.

8.3.1.6 Add 2,0 ml of the aniline (5.4) to flasks D and F. Heat for 30 min on a boiling water-bath to develop the colour.

8.3.1.7 Cool to room temperature, make up to volume with the 2-propanol/hexane mixture (5.1), mix and leave for 1 h.

8.3.1.8 Measure the absorbance of the blank test solution (flask F; 8.3.1.7) against the reference solution (flask E; 8.3.1.5) and the absorbance of the test solution (flask D; 8.3.1.7) against the reference solution (flask C; 8.3.1.3) using the spectrometer (6.2) at the wavelength of maximum absorbance (between 435 and 445 nm).

8.3.1.9 Subtract the absorbance of the blank test solution from that of the test solution to obtain the corrected absorbance.

8.3.2 Determination of total gossypol

8.3.2.1 Transfer the test portion (8.2) into a 50 ml volumetric flask and add 10 ml of solvent B (5.3).

8.3.2.2 Transfer 10 ml of solvent B (5.3) to a second 50 ml volumetric flask (6.3).

8.3.2.3 Heat the two flasks (8.3.2.1 and 8.3.2.2) for 30 min on a boiling water-bath.

Cool to room temperature and make up to volume with the 2-propanol/hexane mixture (5.1). Mix and leave to settle for 10 to 15 min, then filter and collect the filtrates in 50 ml conical flasks (6.4).

8.3.2.4 Transfer, by means of a pipette, 2 ml of the filtrate obtained from the test portion, containing 40 to 200 µg of gossypol, into each of two 25 ml volumetric flasks and 2 ml of the filtrate obtained from the second flask into each of two other 25 ml volumetric flasks.

Make up the contents of one of the flasks containing the test solution and of one flask from the blank test to volume with the 2-propanol/hexane mixture (5.1).

These solutions will be used as reference solutions.

8.3.2.5 Add 2,0 ml of the aniline (5.4) to the remaining test and blank test solutions. Heat for 30 min on a boiling water-bath to develop the colour. Cool to room temperature, make up to volume with the 2-propanol/hexane mixture (5.1), mix and leave for 1 h.

8.3.2.6 Measure the absorbances of the solutions as specified in 8.3.1.8 and calculate the corrected absorbance as specified in 8.3.1.9.

8.4 Number of determinations

Carry out two determinations on the same test sample.

9 Expression of results

9.1 Method of calculation and formula

The free or total gossypol content, expressed in milligrams per kilogram of the product as received, is equal to

$$\frac{A \times 1\,250 \times 1\,000}{a \times m \times V} = \frac{A \times 1{,}25}{amV} \times 10^6$$

where

A is the corrected absorbance (8.3.1.9 or 8.3.2.6);

m is the mass, in grams, of the test portion (8.2);

V is the volume, in millilitres, of the aliquot portion of filtrate taken in 8.3.1.2 or 8.3.2.4;

a is the specific mass absorbance coefficient (62,5 $cm^{-1} \cdot g^{-1} \cdot l$ in the case of free gossypol; 60,0 $cm^{-1} \cdot g^{-1} \cdot l$ in the case of total gossypol).

Take as the result the arithmetic mean of the two determinations (see 8.4) if the condition for repeatability (see 9.2) is met.

9.2 Repeatability

The difference between the results of two determinations, carried out simultaneously or in rapid succession on the same sample, shall not exceed

15 % (relative) of the mean for gossypol contents less than 500 mg/kg;

75 mg/kg (absolute) for gossypol contents not less than 500 mg/kg and not more than 750 mg/kg;

10 % (relative) of the mean for gossypol contents more than 750 mg/kg.

10 Test report

The test report shall show the method used and the result obtained. It shall also mention any operating details not specified in this International Standard, or regarded as optional, together with details of any incidents likely to have influenced the result.

The test report shall include all the information necessary for the complete identification of the sample.

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Amendments are issued to standards as the need arises on the basis of comments. Standards are also reviewed periodically; a standard along with amendments is reaffirmed when such review indicates that no changes are needed; if the review indicates that changes are needed, it is taken up for revision. Users of Indian Standards should ascertain that they are in possession of the latest amendments or edition by referring to the latest issue of 'BIS Catalogue' and 'Standards: Monthly Additions'.

This Indian Standard has been developed from Doc: No. FAD 5 (1671).

Amendments Issued Since Publication

Amendment No.	Date of Issue	Text Affected

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